

In re Patent Application of:

ARENA ET AL.

Serial No. 09/929,833

Filed: August 14, 2001

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robot micro-machining, to chemically immobilize in 2 by 2 cm micro-grids fragments of cDNA (complementary DNA), or DNA reconstructed on the basis of RNA by reverse transcription. In this way, microarrays containing 10,000 individual cDNA elements are formed. The DNA fragment to be analyzed is marked with fluorescent groups so to obtain different types of sensors to immediately distinguish the fragments of DNA by means of the color of the corresponding fluorescent group with which they are treated. In this way, the microarray can be analyzed simultaneously during the hybridisation phase. The micro-grid is read by means of a confocal microscope at the end of the hybridisation phase providing a two-dimensional image in which colored pins, or spots, appear arranged in a grid. The intensity of the various colors and their combinations is directly correlated to the intensity of the light output by fluorescence by the respective probes and to the degree of affinity between the probes and the individual genes deposited on the grid.--

Please replace the heading "Object and summary of the invention" appearing at page 5, line 23, with the following heading:

-- Summary of the Invention --

Please replace the paragraph beginning at page 5, line 33, with the following rewritten paragraph:

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-- This and other objects, features, and advantages in accordance with the present invention are provided by a system for automatically analyzing the images from a DNA chip after hybridisation. --

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Paragraph beginning at line 1 of page 6 has been amended as follows:

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--In essence, according to the currently preferred embodiment, the invention provides for making a system which provides automatic analysis of the images from a DNA chip after hybridization. This is attained by acquiring the images using optical matrix sensors and processing the acquired images using a Cellular Neural Network (CNN). Such a processing is essentially analog and is achieved spatially on the entire development of the microarray matrix.--

Please replace the paragraph beginning at page 6, line 14, with the following rewritten paragraph:

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--According to the currently preferred embodiment of the invention, images are analyzed by means of a computing process which accounts for the physical-chemical rules at the basis of reactions on the microarray.--

Please replace the paragraph beginning at page 6, line 35, with the following rewritten paragraph:

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--More in particular, this invention relates to a system integrated in a monolithic fashion on a semiconductor for automatically analyzing images, such as images from a microarray of the types comprising optical matrix sensors for the acquisition of images and to a high computing power parallel analog processing architecture, based on the implementation of cellular neural network. Moreover, the

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invention provides integration of the entire image acquisition and processing system on a single chip.--

Please replace the paragraph beginning at page 7, line 15, with the following rewritten paragraph:

--FIG. 1 is a schematic view of a system for automatically analyzing images from a DNA chip after hybridisation according to the present invention.

FIG. 2 is a schematic block diagram of a cellular neural network according to the present invention.

FIG. 3 is a more detailed schematic view of portions of a cellular neural network according to the present invention.

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FIG. 4 is a schematic view of an electric circuit associated with the cellular network according to the present invention.

FIG. 5 is a plot of a weighted output value, $h(x)$, as a function of an input signal, x , representative of the values used according to the present invention.

FIG. 6 is top plan view of a DNA chip after hybridisation and splitting thereof into three chromatic components as used according to the present invention.

FIG. 7 is a flow chart of a method of neural network image processing applied to chromatic components of an image read from a DNA chip according to the present invention.

FIGS. 8a-12 illustrate various operations concerning filtering, segmenting, and the morphological operations, which can be implemented in a system according to this invention, can be conducted to isolate useful information with respect to the various sources of noise which could lead to false

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interpretations of the results during the automatic microarray
image analysis process.--

Please replace the heading "Detailed description
of a preferred embodiment of the invention" appearing at page
8, line 10, with the following heading:

--Detailed Description of the Preferred Embodiments--

Please replace the paragraph beginning at page 8,
line 12, with the following rewritten paragraph:

27
--As mentioned above, the solution according to this
invention offers an advantageous alternative with respect to
traditional methods based on the analysis of fluorescence
images generated by means of a DNA chip. In particular, the
solution according to this invention utilizes the class of
arrays (generally two-dimensional) of analog processors known
as cellular neural networks (CNN) and implements a system
which is able to process such images in real time.--

Please replace the paragraph beginning at page 8,
line 30, with the following rewritten paragraph:

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--As further illustrated hereof, the system 20 can
be configured as a cellular neural network (CNN) processing
system, i.e. as an analog, parallel processing system,
preferably integrated in the same chip housing the block 10 in
which the optical sensor is integrated.--

Please replace the paragraph beginning at page 9,
line 1, with the following rewritten paragraph:

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29 --In particular (again with reference to the block diagram in figure 2), in addition to the array of analog cells with optical sensors forming the block 10 in which the optical sensor is integrated, the system preferably comprises a set of analog memories 11 which can co-operate with sensor 10, according to the criteria which are further described below, as well as an input/output circuit 12, which type is generally known.--

Please replace the paragraph beginning at page 9, line 13, with the following rewritten paragraph:

B10 --Preferably, the control logic 13 directly acts on the circuit 12. The same control logic 13 is usually configured so to directly operate on the array 10 by means of an analog/digital converter 14 to which the instructions contained in a program memory 16 selectively flow via a set of digital registers 15 for the configuration of the cellular neural network.

According to another important characteristic of the invention, the system 20 is configured as a cellular neural network which avoids the need to implement analog/digital conversion and/or vice versa of the values of each element or pixel in the image acquired at output of the optical sensor 10, also allowing to implement the microarray image analysis algorithm according to a totally parallel processing criterion. The various operations forming the algorithm are achieved by suitably setting the parameters which are programmed in the configuration registers 15 of the neural network on a case-by-case basis.--

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Please replace the paragraph beginning at page 10, line 8, with the following rewritten paragraph:

B11
-- The circuit model of each cell 100 is shown in the diagram in figure 4, which schematically illustrates the values included in matrixes $A(ij;kl)$ and $B(ij;kl)$ and in the bias coefficient I_{ij} . The values generate, from an input signal, a corresponding output value which is weighted by a function $h(x)$ illustrated in figure 5.--

Please replace the paragraph beginning at page 10, line 18, with the following rewritten paragraph:

B12
--Returning to the block diagram in figure 2, the block 10 essentially consists of a matrix of analog cells whose inputs are the signals corresponding to the optical sensors which read the image I generated in the microarray.

The analog memory 11 is used to store the images and the intermediate processing stages. Conversely, the instructions and the respective parameters are stored in digital form in the memory 16 and in the registers 15 and are applied to the block 10 by means of the converter 14. The control logic 13 synchronizes the image acquisition and processing operations, in addition to the I/O signals to the end user which pass through the block 12.--

Please replace the paragraph beginning at page 11, line 21, with the following rewritten paragraph:

B13
--Obviously, the algorithms to be implemented depend on the type of analysis required by the expert. However, important steps, such as the reduction of the components,

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noise clearing, or the elimination of deformed spots, will need to be performed in any case. The example shows an algorithm which extracts from an image resulting from two red and green fluorescence probes the spots related to three different levels of each color indicating the three different degrees of affinity between the probes and the genes present in the micro-grid.--

Please replace the paragraph beginning at page 11, line 32, with the following rewritten paragraph:

B14

--Figure 6 illustrates an example of image I from a DNA chip after hybridisation. For classification of affinities, analyzing the two chromatic components R (red) and G (green) only will usually suffice. This is because there are no reactions able to generate appreciable levels of the component B (blue), i.e. the third component of the known RGB (Red Green Blue) color model.--

Please replace the paragraph beginning at page 12, line 15, with the following rewritten paragraph:

B15

--In the solution according to this invention, this form of pre-processing can be eliminated and simple two-color sensors, instead of Bayes sensors, can be used as sensors which are selectively sensitive to distinct chromatic components.--

Please replace the paragraph beginning at page 12, line 20, with the following rewritten paragraph:

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--Furthermore, no digitalization operation is required, since a typically analog treatment is implemented. Consequently, applying an image processing sequence with cellular neural networks based on templates, for example according to the process outlined in the flow chart in figure 7, for each of the chromatic components (R and G) processed, will suffice.--

Please replace the paragraph beginning at page 13, line 29, with the following rewritten paragraph:

B17
--All the operations above, including the final logic AND operation, are carried out within the cellular neural network by means of templates, i.e. by means of suitable sets of parameters which are programmed in the network configuration registers (indicated by number 15 in the diagram in figure 2) on a case-by-case basis. The sequence of operations gives rise to a set of intermediate results corresponding to images which can be stored in the analog memory of the system, indicated by number 11 in figure 2.--

Please replace the paragraph beginning at page 16, line 27, with the following rewritten paragraph:

B18
--The second advantage is the high processing speed which allows to process images which can also be large directly on-chip with very short processing times. Such times depend only on the value of the time constant RC of the cells in the cellular neural network and the acquisition time of the optical sensors because no analog/digital conversion (and/or vice versa) is required for the values of each pixel of the image acquired at optical sensor output with respect to the